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S-adenosylhomocysteine and the ratio of S-adenosylmethionine to S-adenosylhomocysteine are not related to folate, cobalamin and vitamin B6 concentrations

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Abstract

Background It is unclear whether homocysteine itself is causal in the pathogenesis of cardiovascular disease. Alternatively or additionally, the association between homocysteine and cardiovascular disease may be because of its metabolic precursor, S-adenosylhomocysteine, or of the ratio of S-adenosylmethionine to S-adenosylhomocysteine. Therefore, it is relevant to know how these moieties are interrelated, and whether, as is the case for homocysteine, they are influenced by blood levels of folate, cobalamin or vitamin B_6.

Design We cross-sectionally studied a population-based cohort of 97 Caucasian subjects aged 60–85 years. Concentrations of homocysteine, S-adenosylhomocysteine, S-adenosylmethionine, folate, cobalamin and vitamin B6 were measured in fasting blood samples.

Results In multiple regression analysis, homocysteine was associated with vitamin B12 (per 50 pmol L^{-1} increase of cobalamin, change in homocysteine, –0·70 mmol L^{-1}; 95% CI, –1·30 to –0·10 mmol L^{-1}) and folate (per 100 nmol L^{-1} increase in erythrocyte folate, change in homocysteine, –0·68 mmol L^{-1}; 95% CI –1·28 to –0·08 mmol L^{-1}). S-adenosylhomocysteine, S-adenosylmethionine and the ratio of S-adenosylmethionine to S-adenosylhomocysteine were not associated with serum folate, cobalamin or vitamin B6, nor with erythrocyte folate. Furthermore, plasma homocysteine showed a negative correlation with the ratio of S-adenosylmethionine to S-adenosylhomocysteine in plasma ($r = -0{\cdot}27$; $P < 0{\cdot}01$) but not in erythrocytes.

Conclusions In contrast to homocysteine, the plasma concentrations of S-adenosylhomocysteine and the ratio of S-adenosylmethionine to S-adenosylhomocysteine were not associated with the folate, cobalamin and vitamin B6 concentrations in the present study. If these precursors in part explain why homocysteine is associated with cardiovascular disease, homocysteine-lowering treatment with B vitamins may be less effective than currently expected, at least in an elderly population.

Keywords Cardiovascular disease, cobalamin, folate, homocysteine, intervention trials, S-adenosylhomocysteine, S-adenosylmethionine, vitamin B_6.
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Introduction

Hyperhomocysteinemia is an independent risk factor for cardiovascular disease [1–3]. This suggests that treatment with folic acid, and possibly cobalamin and vitamin B_6, which decrease plasma homocysteine, may be beneficial [4–9]. Accordingly, large clinical trials have been initiated to test this hypothesis [10], and two randomized, controlled trials with intermediate endpoints have already been published [11,12].

However, it remains unclear whether homocysteine itself is causal in the pathogenesis of cardiovascular disease. Alternatively or additionally, the association between homocysteine and cardiovascular disease may result from its metabolic precursor, S-adenosylhomocysteine [13–16] or from the ratio of S-adenosylmethionine (the precursor of S-adenosylhomocysteine) to S-adenosylhomocysteine [17,18]. This contention is based on the known effects of these moieties on cellular methylation, disturbances of which may lead to endothelial dysfunction and/or cardiovascular disease [13–15,18]. Thus, it is relevant to know how these moieties are interrelated, and how they relate to B vitamin status. Such data, however, are scarcely available [19].

The aim of this study was to investigate, in an elderly population, the associations between, on the one hand, homocysteine, S-adenosylhomocysteine, and the ratio of S-adenosylmethionine to S-adenosylhomocysteine and, on the other hand, blood levels of B vitamins. We did not study associations between these moieties and cardiovascular disease.

Subjects and methods

Subjects

The Hoorn Study is a longitudinal population-based study addressing glucose intolerance and related complications. It was started in 1989 and consisted of Caucasian men and women aged 50–75 years, as described in detail elsewhere [20]. In the year 2000 these subjects were again invited to partake in the study. For the present cross-sectional study we focused only on 97 participants without type 2 diabetes, randomly selected from this population. All participants gave informed consent for this study, which was approved by the local Ethics Committee.

Methods

Subjects were invited to the Hoorn study centre after an overnight fasting period. We measured each subject's blood pressure, weight and height, and calculated their body mass index and waist-to-hip ratio, as previously described [20]. We drew blood samples for the measurement of homocysteine and its precursors: B vitamins, lipids (total cholesterol, high-density-lipoprotein cholesterol, triacylglycerol), creatinine and haematocrit. We estimated creatinine clearance according to the Cockroft-Gault formula [21]. Hypertension was defined according to the criteria of the Dutch Society of General Practitioners that was in use when these data were collected: systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure of ≥ 95 mmHg, and/or the use of antihypertensive medication [22]. Information on pre-existent cardiovascular diseases (myocardial infarction, angina pectoris, stroke and peripheral atherosclerotic disease) was obtained from the subjects using a translated version of the Rose questionnaire from the London School of Hygiene [23].

Sample preparation

All samples were processed within 30 min, stored at –80 °C (except for lipids and creatinine, which were stored at –20 °C), and analyzed within 3 months. After collection, ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood samples were placed on ice for the determination of homocysteine, S-adenosylmethionine and S-adenosylhomocysteine. For S-adenosylmethionine and S-adenosylhomocysteine measurements, we deproteinized the samples immediately by adding 0·625 mL of a 10% perchloric acid solution to 1 mL of plasma and by adding 1 mL of 5% perchloric acid to 1 mL of whole blood, followed by mixing [18]. We added 0·5 mg of ascorbic acid to 0·5 mL of serum for the determination of total folate [18]. For the determination of total folate in erythrocytes, 1 mL of reagent with ascorbic acid, human serum albumin and sodium azide (ACS:180, Chiron Diagnostics, Fernwald, Germany) was added to 50 μL of whole blood.

Determination of homocysteine, S-adenosylmethionine, S-adenosylhomocysteine and total folates

Total plasma homocysteine was determined with an automated fluorescence polarisation immunoassay on an Abbott IMx analyzer (Abbott Laboratories Ltd, IL, USA) (interassay coefficient of variation, CV, 4%) [24]. We used tandem mass spectrometry for the determination of S-adenosylmethionine and S-adenosylhomocysteine in plasma, and the whole blood, as previously described (intra-assay CV, 4% for both determinations; interassay CV, 8% and 6%, respectively) [25]. We measured total folate in the red blood cell haemolysate and serum, and cobalamin in the serum by means of automated chemiluminescence (Chiron Diagnostics ASC:180® Automated Chemiluminescence System). The intraassay and interassay CVs were 4% and 5% for total folate, respectively, and 4% and 5% for cobalamin. Erythrocyte concentrations of the metabolites were calculated by multiplying the difference between plasma or serum and the whole blood values by 100 haematocrit^{-1}. We used high-performance liquid chromatography for plasma vitamin B_6 determination based on precolumn semicarbazone formation and fluorescence detection [26]. The interassay CV was 7%.

Determination of lipids and creatinine

We determined serum total cholesterol, HDL cholesterol and triglycerides by enzymatic techniques (Boehringer-Mannheim, Mannheim, Germany) and serum creatinine by means of the modified Jaffé method.

Statistical analyses

All analyses were performed with SPSS 10·1 for Windows 95 (SPSS, Chicago, IL, USA). Because the distributions of homocysteine, most of its metabolites and B vitamins were positively skewed, we present medians and interquartile ranges of these variables. We performed univariate and multivariate linear regression analyses to determine associations between homocysteine, or one of its precursors, and B vitamins. In univariate regression analyses, unadjusted β coefficients were calculated (model 1). In model 2 we used multivariate regression analyses to calculate β coefficients for each B vitamin after mutual adjustment for the other two B vitamins. The final model [3] was model 2 with additional adjustment for other possible determinants. In all models we regarded homocysteine, S-adenosylhomocysteine, S-adenosylmethionine or the ratio of S-adenosylmethionine to S-adenosylhomocysteine in plasma or erythrocytes as the dependent variable, respectively. We did not use any log-transformed data in the regression analyses because the determinants had a linear relation with each outcome variable. We calculated which folate measurement (total folate in serum or total folate in erythrocytes) had the strongest association with the respective dependent variables in model 1 by log-transforming these measurements of folate status and comparing changes in homocysteine associated with a 10% increase in each folate measure. In models 2 and 3, we added the folate measure with the strongest association with the dependent variable. In model 3, we added age, sex, creatinine clearance, lipids, hypertension, body mass index and waist-to-hip ratio as possible determinants [2,27–30]. Pearson correlation coefficients were calculated to determine the association between homocysteine and S-adenosylmethionine, S-adenosylhomocysteine or the ratio of S-adenosylmethionine and S-adenosylhomocysteine in plasma and erythrocytes, and to show the association after adjustment for age, sex and creatinine clearance.

Results

General characteristics [Table 1]

Hyperhomocysteinemia (homocysteine concentration > 15 μmol L^{-1}) was present in 16·5% of the population [1]. Concentrations of vitamin B_6, cobalamin and folate in serum were below the reference range in four, five and two subjects, respectively. Total folate concentrations in erythrocytes was within the range as described by the assay's manufacturer. The ratio of S-adenosylmethionine to S-adenosylhomocysteine was significantly higher in erythrocytes than in plasma (24·9 vs. 6·24). The ranges of plasma concentrations of S-adenosylmethionine and S-adenosylhomocysteine were comparable with the ranges in three studies of similarly aged subjects [16–18]. The range of S-adenosylmethionine concentrations in erythrocytes was similar to that in two other studies [17,18], and was higher compared with a study by Perna *et al.* [13]. The range of S-adenosylhomocysteine in erythrocytes was somewhat lower than in a previous study [18]. That study was, however, based on a group of only 50, healthy, participants.

Determinants of homocysteine [Table 2]

As expected, the univariate linear regression analyses showed that vitamin B6, cobalamin and folate in serum and erythrocytes had a significant negative association with the homocysteine. Total folate in erythrocytes was the folate measure with the strongest association with homocysteine. When the concentration of folate in erythrocytes increased by 10%, the concentration of the homocysteine decreased by 0·23 μmol L^{-1}, as opposed to 0·13 μmol L^{-1} for folate in serum. In the multivariate regression analyses, folate in erythrocytes and cobalamin remained significant determinants of homocysteine, even after adjustment for possible confounders (model 3). Lipid concentrations, hypertension and waist-to-hip ratio did not influence the regression coefficients of the B vitamins (data not shown). In addition, sex, creatinine clearance and the body mass index were significantly associated with the homocysteine.

Determinants of S-adenosylhomocysteine and the ratio of S-adenosylmethionine to S-adenosylhomocysteine [Table 2]

S-adenosylhomocysteine, S-adenosylmethionine and their ratio in plasma were not significantly associated with folate in the univariate analyses. For the multivariate analyses, the measurement of folate status (plasma or erythrocyte) that was least weakly associated with the dependent variable of interest was used (see Table 2).

Neither in the univariate nor multivariate regression analyses were S-adenosylhomocysteine, S-adenosylmethionine or the ratio of S-adenosylmethionine to S-adenosylhomocysteine in the plasma significantly associated with any B vitamin. In contrast, these variables in plasma were significantly associated with sex, creatinine clearance and body mass index, except for S-adenosylmethionine, which was associated only with the body mass index. Lipid concentrations, hypertension and waist-to-hip ratio did not influence the regression coefficients of the B vitamins (data not shown).

In erythrocytes, no significant association was found between S-adenosylhomocysteine or the ratio of S-adenosylmethionine to S-adenosylhomocysteine on the one hand, and any B vitamin, sex, creatinine clearance or body mass index on the other (data not shown).

Table 1 Characteristics of the participants (n = 97)

	Mean (SD‡), median (IQR*) or percentage	Range
Age (years)	70·5 (6·4)	60–85
Gender (% male)	56·7	
Body mass index	26·3 (3·5)	20·5–42·8
Waist-to-hip ratio		
Men	0·99 (0·09)	0·89–1·50
Women	0·85 (0·07)	0·69–0·96
Systolic blood pressure (mmHg)	143·6 (19·5)	100–195
Diastolic blood pressure (mmHg)	84·6 (11·3)	56–113
Hypertension (%)	41·2	
Cardiovascular disease (%)	23·7	
Plasma or serum value		
Creatinine (μmol L^{-1})	96·7 (14·6)	59–149
Creatinine clearance (mL min^{-1})†		
Men	69·3 (13·1)	37–112
Women	58·6 (12·2)	40–101
Total cholesterol (mmol L^{-1})	5·84 (0·90)	4·0–8·8
HDL-cholesterol (mmol L^{-1})	1·45 (0·40)	0·8–3·4
Triglycerides (mmol L^{-1})	1·3 (0·93–1·7)	0·5–5·0
Homocysteine (nmol L^{-1})		
Men	(9·3–14·4)	5·8–38·5
Women	11·0 (9·8–13·8)	5·7–24·4
Vitamin B_6 (nmol L^{-1})	36·0 (24·5–51·0)	10–455
Cobalamin (pmol L^{-1})	266 (210–318)	22–626
Folate (nmol L^{-1})	15·0 (11·0–19·2)	3·0–45·4
S-adenosylmethionine (nmol L^{-1})	97·3 (83·9–108·0)	53–208
S-adenosylhomocysteine (nmol L^{-1})	15·6 (12·6–18·8)	6·6–32·7
SAM/SAH ratio§	6·24 (5·29–6·99)	3·6–11·2
Erythrocyte value		
Folate (nmol/L)	551 (423–663)	158–1222
S-adenosylmethionine (nmol L^{-1})	3523 (3049–3950)	2376–5107
S-adenosylhomocysteine (nmol L^{-1})	132 (113–164)	71–522
SAM/SAH ratio§	24·9 (20·8–31·8)	4·6–54·4

*Interquartile range.
†Estimated by the Cockcroft-Gault formula.
‡Standard deviation.
§SAM/SAH ratio = ratio of S-adenosylmethionine and S-adenosylhomocysteine; reference ranges.
Vitamin B_6, 17–100 nmol L^{-1}; cobalamin, 156–672 pmol L^{-1}; folate in serum, > 6·5 nmol L^{-1}; folate in erythrocytes, 125–2500 nmol L^{-1}; S-adenosylmethionine in plasma 34·8–79·5 nmol L^{-1}; S-adenosylmethionine in erythrocytes 2701–5344 nmol L^{-1}; S-adenosylhomocysteine in plasma 11·8–39·4 nmol L^{-1}; S-adenosylhomocysteine in erythrocytes 128–328 nmol L^{-1} (generally accepted reference ranges do not exist for S-adenosylmethionine and S-adenosylhomocysteine).

Correlations among homocysteine, S-adenosylmethionine, S-adenosylhomocysteine and their ratio [Table 3]

Homocysteine had a significant negative correlation with the ratio of S-adenosylmethionine to S-adenosylhomocysteine in plasma, but not in erythrocytes. S-adenosylmethionine and S-adenosylhomocysteine in plasma, but not in erythrocytes, were strongly correlated (r = 0·68; P < 0·001) Adjustment for age, sex and creatinine clearance did not materially affect any of these univariate correlations (data not shown). Figure 1 depicts the relations between S-adenosylmethionine and S-adenosylhomocysteine in plasma and in erythrocytes, and between the homocysteine level and the ratio of S-adenosylmethionine to S-adenosylhomocysteine in plasma.

Discussion

This population-based study is the first to show that, in contrast to homocysteine, plasma concentrations of S-adenosylhomocysteine and the ratio of S-adenosylmethionine to S-adenosylhomocysteine are not associated with concentrations of folate, cobalamin and vitamin B_6 in an elderly population. Furthermore, our results showed that, in this elderly population, creatinine clearance, body mass index and sex were determinants not only of homocysteine, but also of S-adenosylhomocysteine, and the ratio of S-adenosylmethionine to S-adenosylhomocysteine in plasma. Homocysteine showed a negative correlation with the ratio of S-adenosylmethionine to S-adenosylhomocysteine in plasma, but not in erythrocytes. In fact, the erythrocyte concentrations of S-adenosylmethionine and

Table 2 Linear regression analyses with homocysteine, S-adenosylhomocysteine, S-adenosylmethionine or the ratio of S-adenosylmethionine to S-adenosylhomocysteine *in plasma* as the dependent variable

	Homocysteine beta* (95% CI)	SAH beta* (95% CI)	SAM beta* (95% CI)	SAM/SAH ratio beta* (95% CI)
Model 1				
Vitamin B_6 (per 10 nmol L^{-1})	−0·22 (−0·42 to −0·02)	−0·12 (−0·34–0·10)	0·11 (−0·93–1·15)	0·06 (−0·01–0·12)
Cobalamin (per 50 pmol L^{-1})	−0·75 (−1·35 to −0·15)	0·14 (−0·46–0·74)	2·47 (−0·53–5·47)	0·08 (−0·12–0·28)
Folate serum (per 10 nmol L^{-1})	−1·76 (−3·10 to −0·42)	−0·76 (−2·26–0·74)	0·44 (−6·67–7·54)	0·34 (−0·08–0·76)
Folate erythrocytes (per 100 nmol L^{-1})	−0·83 (−1·43 to −0·23)	0·01 (−0·59–0·61)	0·62 (−2·18–3·42)	−0·01 (−0·21–0·19)
Model 2				
Vitamin B_6 (per 10 nmol L^{-1})	−0·14 (−0·54–0·26)	−0·10 (−0·38–0·18)	−1·68 (−3·90–0·54)	0·04 (−0·04–0·12)
Cobalamin (per 50 pmol L^{-1})	−0·65 (−1·25 to −0·05)	0·29 (−0·41–0·99)	2·78 (−0·42–5·98)	0·02 (−0·18–0·22)
Folate (per 10 or 100 nmol L^{-1})†	−0·70 (−1·30 to −0·10)	−5·30 (−23·5–12·90)	1·78 (−1·42–4·98)	1·72 (−3·28–6·72)
Model 3				
Vitamin B_6 (per 10 nmol L^{-1})	−0·21 (−0·61–0·19)	−0·08 (−0·32–0·16)	−1·12 (−3·34–1·10)	0·04 (−0·02–0·10)
Cobalamin (per 50 pmol L^{-1})	−0·70 (−1·30 to −0·10)	0·40 (−0·20–1·0)	1·81 (−1·59–1·10)	0·08 (−0·28–0·13)
Folate (per 10 or 100 nmol L^{-1})†	−0·68 (−1·28 to −0·08)	−5·60 (−21·20–10·0)	2·0 (−1·20–5·20)	2·11 (−1·89–6·11)
Age (per 10 years)	−1·79 (−3·79–0·21)	0·54 (−1·50–2·58)	1·91 (−8·97–12·79)	−0·07 (−0·59–0·45)
Sex (female vs. male)	−2·52 (−4·81 to −0·23)	−5·11 (−7·38 to −2·83)	5·74 (−6·63–18·12)	2·15 (1·56–2·74)
Creatinine clearance (per 10 mL min^{-1})	−1·7 (−2·84 to −0·56)	−2·49 (−3·65 to −1·33)	−3·21 (−9·37–2·95)	0·65 (0·35–0·95)
Body mass index (per 1 kg m^{-2})	0·39 (0·02–0·75)	0·71 (0·34–1·08)	2·25 (0·30–4·21)	−0·11 (−0·15 to −0·07)

CI, confidence interval; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SAM/SAH, S-adenosylmethionine to S-adenosylhomocysteine ratio.

Model 1: univariate regression analyses; Model 2: multivariate regression analyses; Model 3: as Model 2 with additional adjustment for age, sex, creatinine clearance and body mass index.

*Change in concentration of homocysteine, SAH, SAM and SAM/SAH per increment of each determinant.

†In the analysis with homocysteine and S-adenosylmethionine we added folate to the erythrocytes (expressed per 100 nmol L^{-1}) and in the analysis with S-adenosylhomocysteine or SAM/SAH we added folate to the serum (expressed per 10 nmol L^{-1}).

Table 3 Correlation coefficients among plasma homocysteine and S-adenosylhomocysteine, S-adenosylmethionine and the ratio of S-adenosylmethionine to S-adenosylhomocysteine in plasma and erythrocytes

	Plasma			Erythrocytes		
	SAH	SAM	SAM/SAH ratio	SAH	SAM	SAM/SAH ratio
Homocysteine	0·14	−0·08	−0·27*	0·003	0·08	0·08
SAH plasma		0·68**		0·05	0·1	
SAH erythrocytes					−0·04	
SAM erythrocytes		−0·012				

*$P < 0·01$; **$P < 0·001$. *P*-value of the Pearson correlation coefficient.
SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SAM/SAH, ratio of S-adenosylmethionine and S-adenosylhomocysteine.

S-adenosylhomocysteine were not associated with any of the variables studied.

Folate in erythrocytes and serum cobalamin, but not vitamin B_6 in plasma, were independently associated with plasma homocysteine, but not with S-adenosylhomocysteine or the ratio of S-adenosylmethionine to S-adenosylhomocysteine in plasma or erythrocytes. The association between homocysteine and folate and cobalamin in elderly subjects has been reported previously [31,32]. However, in the Framingham study, plasma vitamin B_6 also was an independent significant determinant [31]. In several other studies, the association between homocysteine and vitamin B_6 disappeared after adjustment for cobalamin [6,8,9].

Poirier *et al.* showed, although in a younger age group, that *dietary* folate and vitamin B_6 did not correlate with erythrocyte values of the ratio of S-adenosylmethionine to S-adenosylhomocysteine after adjustment for body weight and age. Cobalamin was not measured in this study [19]. In apparent contrast to these studies, Perna *et al.* reported that in patients on haemodialysis, oral therapy with methyltetrahydrofolate, the active form of folic acid decreased homocysteine concentration and increased the S-adenosylmethionine to S-adenosylhomocysteine ratio [33]. The strength of baseline correlations between B-vitamins and homocysteine concentration and the S-adenosylmethionine to S-adenosylhomocysteine ratio was, unfortunately, not reported. Another point to be made about Perna's observations is that renal insufficiency is characterized by marked folate resistance [34], and the findings may thus not apply to subjects without significant renal disease. In conclusion, it appears difficult to predict whether supplementation with vitamin B_6, cobalamin or folate will influence concentrations of S-adenosylhomocysteine, or the ratio of S-adenosylmethionine to S-adenosylhomocysteine in healthy subjects or vascular patients. This remains to be determined in experimental studies of increased B vitamin intake.

Creatinine clearance was an important determinant of homocysteine, S-adenosylhomocysteine, and the ratio of S-adenosylmethionine to S-adenosylhomocysteine in plasma. An independent relation between homocysteine and renal function is well established, although its mechanism is still debated [32,35–37]. An association with creatinine clearance has been reported once, although in univariate analysis only, for plasma S-adenosylhomocysteine [18]. This may be explained by the fact that S-adenosylhomocysteine can be removed from plasma by the kidney [38,39].

Sex and body mass index were also significantly associated with plasma concentrations of homocysteine, S-adenosylhomocysteine and the ratio of S-adenosylmethionine to S-adenosylhomocysteine. This has been demonstrated before for homocysteine only [8,32]. Plasma S-adenosylhomocysteine and the ratio of S-adenosylmethionine to S-adenosylhomocysteine may be associated with body mass index and sex by the same (largely unexplained) mechanisms as homocysteine, for example through physical activity, insulin sensitivity and sex hormones [28,40].

Finally, it appeared that S-adenosylhomocysteine and S-adenosylmethionine correlated in plasma, but not in erythrocytes. Furthermore, the plasma and erythrocyte values of S-adenosylhomocysteine and S-adenosylmethionine did not correlate. Homocysteine concentration correlated only with the ratio of S-adenosylmethionine to S-adenosylhomocysteine in plasma. There is a disturbing lack of insight into the regulation of metabolic fluxes of S-adenosylmethionine and S-adenosylhomocysteine from cells to plasma. Concentrations of S-adenosylmethionine are probably lower in erythrocytes than in other cells, as erythrocytes are the only type of cells that cannot remethylate homocysteine [41]. One option is that S-adenosylmethionine 'leaks' from erythrocytes to plasma. However, we would then expect a correlation between S-adenosylmethionine in erythrocytes and plasma, which we did not find. Second, it has been suggested that S-adenosylmethionine may be exported from the liver, which has a high methionine metabolism, for use in other tissues [42]. A third possibility is that S-adenosylmethionine is exported from intestinal cells after adenosylation of food-derived methionine. However, there is no direct evidence supporting the latter concept. S-adenosylhomocysteine may exit from the cell when the binding capacity to intracellular proteins is exceeded [39]. However, the absence of a correlation between the erythrocyte and plasma concentrations of S-adenosylhomocysteine in our study argues against this theory. Finally, it is unknown whether S-adenosylmethionine can be transmethylated to S-adenosylhomocysteine in plasma. The correlation we found between these variables in the plasma would support this possibility. In summary, the regulation of metabolic

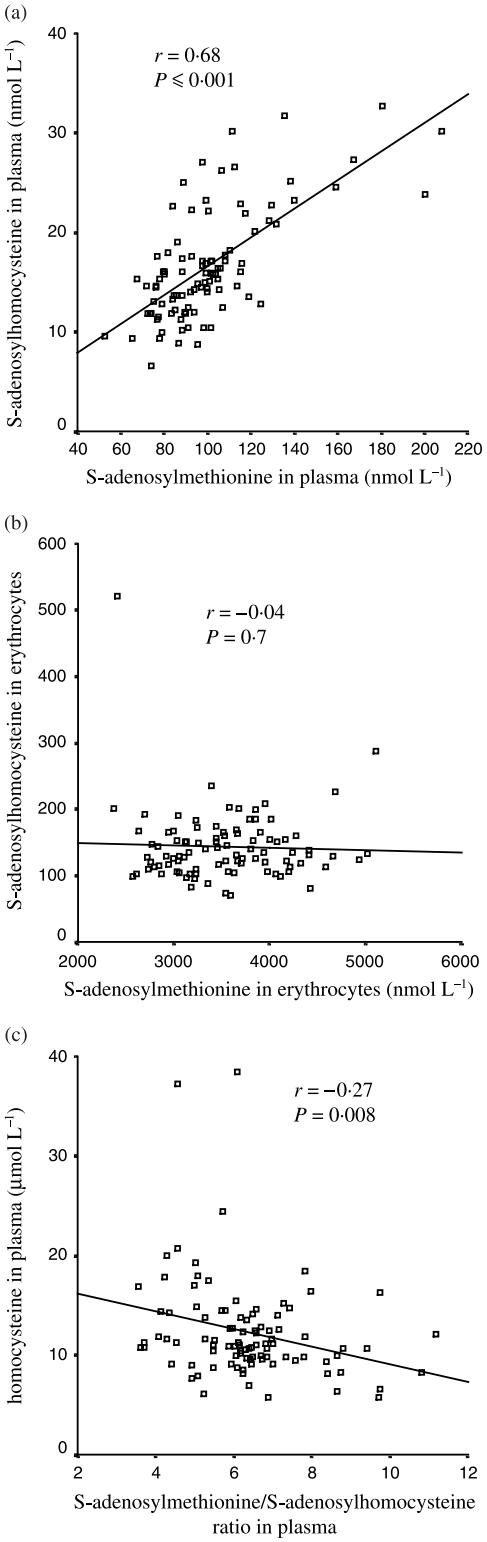

Figure 1 Scatterplot of (a) S-adenosylmethionine and S-adenosylhomocysteine in plasma, (b) S-adenosylmethionine and S-adenosylhomocysteine in erythrocytes, and (c) the ratio of S-adenosylmethionine to S-adenosylhomocysteine and homocysteine in plasma; *r*, Pearson correlation coefficient; *P*-value corresponding to the Pearson correlation coefficient.

fluxes of S-adenosylmethionine and S-adenosylhomocysteine is probably multifactorial. Further research is necessary to study these mechanisms.

It is not fully clarified whether homocysteine, S-adenosylhomocysteine and (or) the ratio of S-adenosylmethionine to S-adenosylhomocysteine is causal in the pathogenesis of cardiovascular disease. Likewise, it is unknown whether intracellular or plasma levels of S-adenosylmethionine and S-adenosylhomocysteine are most relevant for vascular damage. Recent studies suggest an important role for S-adenosylhomocysteine [14–16]. Dayal *et al.* demonstrated that the development of endothelial dysfunction in mice is associated with increased concentrations of S-adenosylhomocysteine and a decreased ratio of S-adenosylmethionine to S-adenosylhomocysteine in liver and brain [15]. Lee *et al.* showed an inhibitory effect of S-adenosylhomocysteine on the regeneration of vascular endothelial cells, which predisposes to atherosclerosis [14]. Furthermore, it has been reported that in patients with peripheral arterial occlusive disease, S-adenosylhomocysteine in plasma and in erythrocytes is increased and the ratio of S-adenosylmethionine and S-adenosylhomocysteine is decreased [18]. In erythrocytes of patients with uraemia, similar findings have been reported [13]. If S-adenosylhomocysteine, or the ratio of S-adenosylmethionine to S-adenosylhomocysteine indeed prove to be causal, at least in part, the effect of B vitamin supplementation on cardiovascular disease may turn out to be smaller than expected, as the B vitamin concentrations were unrelated to these variables in the present study.

This study had some limitations. In the first place, the study population consisted of individuals aged 60–85 years, all Caucasians. We do not know whether our findings can be generalized to younger individuals or other ethnic groups. However, there are two advantages in studying this particular age group. First, low to low-normal vitamin B_6, cobalamin and folate concentrations are common in the elderly [43–45]. Second, the homocysteine concentration increases with age [8]. Therefore, wider ranges of B vitamins and homocysteine concentrations can be studied in the elderly. Another limitation may be that we only measured fasting homocysteine concentrations. Thus, we do not know whether the findings also apply to nonfasting or postmethionine homocysteine concentrations. Finally, the precision of creatinine clearance estimation by the Cockroft-Gault formula is inherently limited, which may have resulted in an underestimation of the strength of the correlations with renal function.

In conclusion, as the B vitamin concentrations were not determinants of S-adenosylhomocysteine or the ratio of S-adenosylmethionine to S-adenosylhomocysteine in the present study, it is uncertain whether B-supplements will lower these possibly harmful intermediates of homocysteine metabolism. If these precursors in part explain why homocysteine is associated with cardiovascular disease, homocysteine-lowering treatment with B vitamins may be less effective than currently expected.

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